

Age, Growth, Food Habits, and Reproduction of the  
Redline Darter *Etheostoma rufilineatum* (Cope)  
(Perciformes: Percidae)  
in Virginia

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**ABSTRACT.**— Life history aspects of *Etheostoma rufilineatum*, the redline darter, were investigated from May 1981 to May 1982 in the North Fork Holston River, Virginia. Analysis of scale samples indicated that males and females grew at approximately the same rate, but males reached a greater maximum length. Estimated annual survival rate for age II and older males was 0.28 and for females 0.03. Aquatic insect larvae were the major food items, and dipterans predominated numerically (68-87%) year-round. Feeding over a 24-hour period peaked from early to late afternoon (1600-2000 hr). The sex ratio favored males throughout the year (2.5:1) and was attributed to differential survival and distribution. Age I fish more than 42 mm long, of both sexes, were sexually mature. Ripe males were first collected in March, although spawning coloration was evident in December. Female ovaries began maturing in late February, and spawning occurred from mid-May to mid-August.

### INTRODUCTION

The darters are small members of the family Percidae and constitute a diverse group of North American fishes, with 145 species in 3 genera and 28 subgenera (Collette 1967; Page 1983). They reach maximum lengths of 35-200 mm and differ widely in morphological characteristics, habitat preference, and behaviors. A comprehensive review of the biology and ecology of darters was published by Page (1983).

The redline darter, *Etheostoma rufilineatum* (Cope), is one of 13 species in the subgenus *Nothonotus*, one of the more gaudy groups of darters. *Nothonotus* species exhibit strong sexual dimorphism. Males of most species display brilliant coloration during most of the year. The species occur in riffle habitats of clear upland streams. Most occupy the

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Ohio River basin, but one species occurs in direct tributaries of the lower Mississippi River, one in the Mobile Bay drainage, and two in the Ozarks. Of the 13 species, life history studies have been conducted only on *Etheostoma acuticeps* Bailey, the sharphead darter (Jenkins and Burkhead 1975; Bryant 1979) and *Etheostoma maculatum* Kirtland, the spotted darter (Raney and Lachner 1939), and comparative ecological studies were done on 3 species (Stiles 1972).

The redline darter occurs in tributaries of the Tennessee and Cumberland rivers in Virginia, North Carolina, Tennessee, Kentucky, Georgia, Mississippi, and Alabama (Etnier 1980). It is found in swift, shallow riffles of clear streams and may exist in riffles shallower than those preferred by other *Nothonotus* species (Zorach 1970). Except for studies on systematics (Zorach 1970) and breeding and food habits of three *Nothonotus* species (Stiles 1972; Bryant 1979), no biological information is available on the redline darter. The present study was conducted to describe age, growth, food habits, and spawning of a population in Virginia.

## MATERIALS AND METHODS

### STUDY AREA

Field sampling was conducted on the North Fork Holston River, a fourth-order stream in the Ridge and Valley Province of southwestern Virginia. The study site was a 350 m section at River Mile 86.9 (36°55'N, 81°40'W), about 8 km upstream from Saltville, Smyth County. The river at this site averages 29 m wide and consists primarily of riffle habitat with cobble and boulder substrate. Water temperatures, recorded daily with a Ryan 30-day thermograph, ranged from 1°C in February to 29°C in July. Water quality characteristics, collected monthly and analyzed with a Hach DR-EL/1 field kit, are summarized in Table 1. Detailed water quality data for the North Fork Holston River were compiled by Poppe (1982). A total of 41 fish species occurs at the study site (Widlak 1982).

### FISH COLLECTIONS

Redline darters were collected twice monthly from May to August 1981, and monthly from September 1981 to May 1982. Sampling was done exclusively with a Coffelt BP-1 backpack electroshocking unit with direct current output, and dip nets. Waterscopes were used to facilitate sighting of darters during high water levels. All habitats were electrofished to obtain representative samples of redline darters, and an attempt was made to collect at least 10 darters on each sampling date. Seventeen to one-hundred twenty-six specimens were collected per sample during summer and fall sampling, but only three to seventeen were collected during the winter months because of high water levels or ice conditions. Specimens were placed on ice to reduce regurgitation of

Table 1. Water chemistry characteristics collected monthly at North Fork Holston River Mile 86.9, January 1981-March 1982.

Characteristic	Mean	Range	N
Hardness (mg/l)	130	90-175	12
Alkalinity (mg/l)	116	70-160	14
pH	7.1	6.6-8.3	12
Dissolved oxygen (mg/l)	9	7-12	12
Turbidity (FTU)*	14	0-25	11
Conductivity ( $\mu$ mhos)	143	81-218	12

\* FTU = Formazin turbidity units

stomach contents before preservation in 10% buffered formalin. Preservation produced a mean shrinkage of 2 mm in length and gain of 0.2 g in weight. In the laboratory, the preserved fish (499 males, 183 females) were measured (total lengths to nearest 1.0 mm) and weighed (to nearest 0.1 g).

#### AGE AND GROWTH

Scale samples from 126 males and 65 females were taken from the left side above the lateral line and below the spiny dorsal fin at the tip of the depressed pectoral fin, mounted on optical plastic slides, and examined under a compound microscope at 10X magnification. Measurements were made with an ocular micrometer from the center of the focus to each annulus and to the scale margin in the anterolateral field. Length-frequency distributions were plotted as a check on scale readings. Age and growth data for males and females were analyzed using a computer program developed by Marques et al. (1982). Regressions for body length-scale radius relation were fitted by linear regression and length-weight relations were computed. Growth curves were fitted to the von Bertalanffy growth equation (Ricker 1975):

$$L_t = L_\infty (1 - e^{-K(t-t_0)})$$

where  $L_t$  = total length (mm) at time  $t$ ,  $L_\infty$  = asymptotic length (mm),  $K$  = growth coefficient,  $t$  = time (age), and  $t_0$  = hypothetical age at zero length. Annual survival of males and females was computed by the unbiased minimum variance estimator of Chapman and Robson, which is based on coded ages and the frequency of individuals in each age class (Everhart et al. 1975). Since age 0 and I fish were less vulnerable to the sampling gear, they were not included in these survival estimates.

#### FOOD HABITS

Ten darters per month, sampled randomly from collections made during each sampling period, were dissected to determine seasonal food



habits. If fewer than 10 fish were collected in a month, all specimens were examined. Stomach contents were removed, sorted, identified to order or family (Hilsenhoff 1975; Merritt and Cummins 1978; Barnes 1980), and counted. Sampling to determine feeding chronology was conducted on 2 July, 13 August, and 9 September 1981. Twelve to twenty-four darters were collected during each of six 4-hour intervals (1200, 1600, 2000, 2400, 0400, and 0800). Stomach contents were pooled for each sampling time, blotted on a paper towel, and volumetric displacement was measured with a 1 cc plastic syringe. Mean stomach volume for each time interval was computed. These 24-hour samples (329 fish) were also included in seasonal food habits analyses.

#### REPRODUCTION

The reproductive cycle of the male was studied by recording the size and appearance of testes of 427 males, and by external body coloration. Ovaries and digestive tracts of females were removed, and the ovaries weighed to the nearest 0.01 g. Adjusted body weights (body weight after removal of stomach, ovaries, intestinal tract, and liver) of females were also obtained. Ovaries were examined under a dissecting microscope and classified as (1) gravid - containing maturing eggs; (2) ripe - containing ripe eggs; (3) spent - containing some ripe eggs and showing apparent resorption; and (4) resting - containing no mature or maturing eggs. All eggs in both ovaries were counted and categorized as (1) mature (ripe) - largest in size, translucent, indented, and containing a single large oil globule; (2) maturing - intermediate in size, opaque, and yellow; and (3) immature - smallest in size, round, and white. Samples of 10 mature and maturing eggs from each pair of ovaries were measured to the nearest 0.1 mm with an ocular micrometer. A gonosomatic index (GSI) was calculated for females by multiplying the ovary weight by 1000 and dividing by the adjusted body weight (Burr and Page 1978, 1979); values were then plotted over time. No female darters were collected in January and only two in December; consequently, these months were not represented in the GSI computations. Analysis of covariance tested for homogeneity between ovary weight and body weight, total length,  $\log_{10}$  body weight, and cube of total length (de Vlaming et al. 1982). Relations between ova diameter, number of mature and maturing ova, and total length of female, and ova number and adjusted body weight in pre-spawning fish were computed by simple linear regressions. Attempts to observe spawning at the study site and in the laboratory, and to rear darters for larval descriptions, were unsuccessful.

#### RESULTS AND DISCUSSION

The redline darter was the predominant darter and one of the most abundant fish species at the study site. A total of 682 was collected

during the study. Large males (longer than 50 mm) occurred consistently in the swiftest sections of riffles; smaller males and females were collected in swift riffles during summer, but were found frequently in areas with moderate current or along the margins of riffles in other seasons. Young-of-the-year darters were not well represented in samples, and were generally found in protected areas with low current velocity, adjacent to emergent vegetation or streamside brush. Larval darters apparently drift passively into pool areas (Scalet 1973) and have been found at depths of 3 meters (Stiles 1972). The habitat of redline darters in the North Fork Holston River concurs with that reported for other *Nothonotus* species (Raney and Lachner 1939; Raney and Suttkus 1964; Zorach 1969, 1970).

#### AGE AND GROWTH

Scale radius and total length of fish were strongly correlated ( $r = 0.85$ , females;  $r = 0.93$ , males). Regenerated scales were common on fish of all sizes, but at least three readable scales were available from each fish collected. Annuli were recognized by crowding of circuli in the anterior field and cutting over in the lateral field. Annulus formation was in early to mid-March at water temperatures of  $5^{\circ}$  to  $20^{\circ}\text{C}$ , as is typical of other darter species (Fahy 1954; O'Neil 1981; Shute et al. 1982). The body-scale relations for males and females were linear. Equations for the fitted regression lines were as follows:

$$\begin{array}{ll} \text{Males} & L = 14.3 + 0.619(S) \quad (R^2 = 0.859) \\ \text{Females} & L = 10.0 + 0.708(S) \quad (R^2 = 0.720) \end{array}$$

where  $L$  = total length (mm) and  $S$  = scale radius magnified (focus to margin in the lateral field in mm). Back-calculated lengths, based on scale measurements, approximated actual lengths of darters at capture for all age classes (Table 2). Length-frequency distributions for males and females did not provide an adequate indication of age class structure. Scale readings were assumed to be accurate because a random sample of 30 scales was aged, with few discrepancies, by several fishery biologists. Young-of-the-year darters appeared as an identifiable age class in June, but overlap between lengths of 40 to 60 mm obscured separation of the intermediate age classes (I and II). Scale analysis indicated that maximum age for males and females was four years and three years, respectively. Chapman-Robson estimates for annual survival were  $0.28 (\pm 0.003)$  for males and  $0.03 (\pm 0.001)$  for females.

Growth of redline darters was rapid and uniform for all age classes. Because only one age III female (67 mm) was collected, length-at-age data were insufficient to obtain a reliable growth equation for females. The von Bertalanffy growth parameters for males were as follows:

$$L_{\infty} = 88 \text{ mm}, t_0 = -0.815 \text{ years}, K = 0.378$$

Table 2. Actual and back-calculated lengths (linear regression) at capture for redline darters, North Fork Holston River, May 1981-May 1982.

Sex and age	N	Actual length (mm)	Back-calculated (mm)
Males			
I	54	43.6	44.8
II	49	58.1	56.9
III	19	65.7	65.7
IV	4	76.7	75.6
Females			
I	34	41.9	43.0
II	30	54.7	53.3
III	1	67.0	65.2

Table 3. Numerical abundance and percent of total number of food items for different categories of food in 546 redline darter stomachs, 1981-1982.

Food	Dec-Feb (N=10)		Mar-May (N=36)		Jun-Aug* (N=377)		Sep-Nov (N=123)	
	No.	%	No.	%	No.	%	No.	%
Diptera								
Chironomidae	120	86.3	208	65.6	1665	59.8	855	62.9
Simuliidae	1	0.7	25	7.9	203	7.3	171	12.6
Other	-	-	5	1.6	36	1.3	11	0.8
Ephemeroptera								
Baetidae	5	3.6	19	6.0	212	7.6	75	5.5
Heptageniidae	1	0.7	8	2.5	118	4.2	25	1.8
Siphonuridae	-	-	11	3.5	98	3.5	55	4.0
Other	1	0.7	7	2.2	4	0.1	5	0.4
Trichoptera								
Hydropsychidae	2	1.4	5	1.6	130	4.7	79	5.8
Hydroptilidae	6	4.3	6	1.9	130	4.7	14	1.0
Other	-	-	5	1.6	29	1.0	11	0.8
Plecoptera	-	-	-	-	5	0.1	-	-
Coleoptera	-	-	-	-	8	0.2	3	0.3
Hydracarina	3	2.2	16	5.1	122	4.4	56	4.1
Nematomorpha	-	-	2	0.6	-	-	-	-
Empty stomachs	1	10.0	2	5.6	37	9.8	14	11.4
Totals	139	100	317	100	2784	100	1360	100

\* Crayfish, fish eggs, and unidentified invertebrate eggs were collected in a few stomachs during this period.



Males grew rapidly during the first two years of life and slowed thereafter. The largest male collected was 80 mm (age IV), approaching the estimated maximum length of 88 mm for males. Length-weight relations for male and female redline darters were described by the following regression equations:

$$\text{Males } \log_{10} W = -5.42 + 3.32 \log_{10} L \quad (R^2 = 0.987)$$

$$\text{Females } \log_{10} W = -5.11 + 3.14 \log_{10} L \quad (R^2 = 0.956)$$

where  $W$  = weight (g), and  $L$  = total length (mm). Both sexes grew at similar rates.

#### FOOD HABITS

Contents of 546 stomachs were identified and tabulated (Table 3). Empty stomachs made up only 9.9% (range, 5.6 to 11.4%) of the total sample by season (Table 3). Dipteran larvae were the dominant food items throughout the year. Chironomidae and Simuliidae made up from 67.1% of the diet in June-August to 87.0% in December-February, and occurred in 10.0 to 83.3% of the stomachs examined monthly. Ephemeroptera and Trichoptera larvae were important items in summer and fall; 9.8 to 29.3% of stomachs examined from June to November contained these larvae. Hydracarina were eaten throughout the year, but were most common in stomachs in March to May (5.1% of the diet). Food of young-of-the-year was not determined, but may have been largely zooplankton, as reported for the young of other species (Scalet 1972; Burr and Page 1978, 1979; Page 1980). The food of juveniles 25 to 40 mm was similar to that of adults. Items found infrequently in stomachs (less than 1% of the diet) included Plecoptera, Coleoptera, Nematomorpha, and unidentifiable invertebrate eggs. Fish eggs and small crayfish (Decapoda) were found in the stomachs of several large males (60+ mm).

Sample sizes for the 24-hour feeding studies on 2 July, 13 August, and 9 September were 95, 126, and 98 fish, respectively. Stomach content analyses indicated a distinct feeding chronology and similar feeding patterns on all three sampling dates (Fig. 1). Mean food volumes in stomachs increased from mid-morning through late afternoon and early evening, and decreased from late evening to early morning. Feeding peaks were at 2000 hr on 2 July (0.021 ml/stomach) and 9 September (0.028 ml/stomach), and at 1600 hr on 13 August (0.027 ml/stomach). None of the fish collected at 1600 hr on the three dates had empty stomachs, but 18 to 30% of those collected at 2400 hr and 76 to 78% collected at 0400 hr were empty. These observations suggest that redline darters are diurnal sight feeders. Scalet (1972) reported that *Etheostoma radiosum* when feeding may rely on visual cues, particularly on movements of prey, and similar visual feeding has been proposed for other darter species (Mathur 1973; Adamson and Wissing 1977; Schenck and Whiteside 1977).

## REPRODUCTION

Darters less than 35 mm long could not be sexed by external characteristics or by gonadal examination and were considered juveniles. Adult males and females were easily distinguished by their sexual dichromism. Males in breeding condition were readily distinguishable from non-breeding males by body coloration. Non-breeding males lacked coloration on fins and pelvic region and were similar in appearance to non-breeding females. Their testes were small and translucent. In contrast, the vertical fins of breeding males were edged with a band of dark green and inner bands of white and bright red. The basal portion of these fins was dusky gray. Paired fins were yellow or greenish-yellow basally and edged with bright red. The pelvic region was dark green and the abdomen creamy white to yellow. Body coloration varied from light tan to dark brown, with bright red lateral spots. Testes were enlarged, creamy white, and opaque. Bright coloration was apparent on males throughout the year, but enlargement of testes was not observed until March. Males in spawning condition were collected throughout spring and summer, but testes were reduced in size in all fish collected in September. About 72% of age I males, all those longer than 40 mm, were sexually mature.

Females were less colorful although breeding females were generally darker than others. They lacked the bright coloration on the fins and body; vertical and paired fins were heavily spotted with black. The body was darker than that of the male and lacked red spots, but the caudal base of both sexes had prominent white spots. All females collected between September and mid-February had resting ovaries, and ova development first became apparent in March. By May the ovaries contained both maturing and fully ripe ova. Females also reached sexual maturity at age I; 56% of age I females were in spawning condition. The smallest ripe female (42 mm long) was collected in July 1981.

Sex ratios of redline darters at the study site were strongly skewed in favor of males. The overall ratio of males to females was 2.7:1, a significant deviation from a 1:1 sex ratio ( $\chi^2 = 146.4$ ,  $p < 0.005$ ). Seasonal differences or sampling biases may have contributed to the apparent temporal changes in distribution of the sexes. During summer, the sex ratio averaged 2.5:1, since both sexes were present in riffles for spawning. As females moved out of shallow riffle areas after spawning, the sex ratio increased to 5:1 (September) and then returned to 2.5:1 as age 0 individuals were recruited into the population. It is likely that electrofishing was selective for males. Darters shocked in swift riffles were swept into the water column and easily netted, while those in other areas often remained on the bottom and were more difficult to collect.

Total egg complements of 85 female redline darters ranged from 50



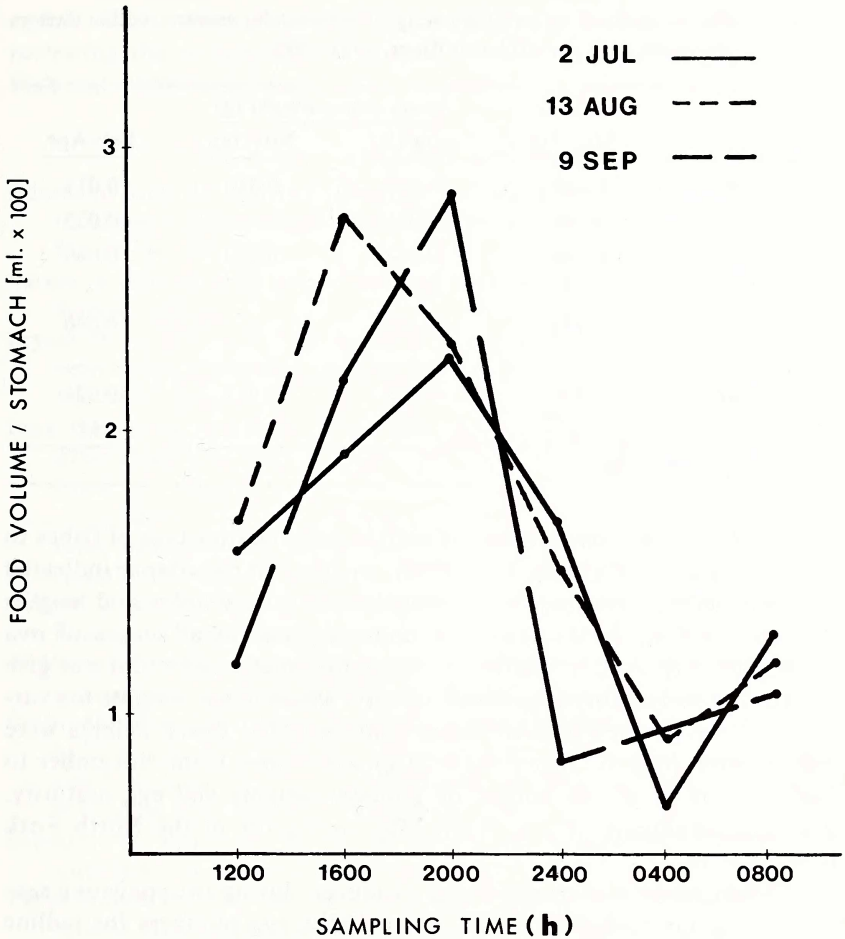


Fig. 1. Twenty-four hour feeding chronologies for redline darters in the North Fork Holston River, 2 July, 13 August, and 9 September 1981.

to 331; numbers of maturing (diameter 0.7-1.5 mm) and mature (1.6-2.2 mm) eggs combined ranged from 23 to 131. Diameter of mature ova and total length of female were not significantly correlated ( $r = 0.08$ ). The number of mature ova and total length of female, and the number of mature ova and adjusted body weight, were slightly correlated ( $r = 0.498$  and  $0.537$ , respectively).

Spawning occurred between May and August 1981 at water temperatures of  $14^{\circ}$  and  $26^{\circ}\text{C}$ . Ripe females were collected as early as 3 May and as late as 18 August. The gonosomatic index (GSI) was not used to identify the spawning season of redline darters because previous

Table 4. Comparison of mean ovary weights of sexually mature redline darters in the North Fork Holston River, 1981-1982.

Size class (mm)	May-Jul	Mean ovary weight (g)		
		Aug-Oct	Nov-Jan	Feb-Apr
40-44	0.054	0.012	0.010	0.011
45-49	0.086	0.031	-	0.035
50-54	0.146	0.048	0.020	0.060
55-59	0.145	0.061	-	-
60+	0.174	0.110	-	0.080
Mean	0.118*	0.042	0.017	0.026
N	62	85	3	11

\*Significant at  $p = 0.05$ .

statistical analyses have shown the ovary-body relationships of fishes to be misleading (de Vlaming et al. 1982). Analysis of covariance indicated that relationships between ovary weights and body weights and lengths in female redline darters were not homogeneous for all stages of ova development ( $p < 0.001$ ); therefore, the gonosomatic index did not give an accurate indication of gonadal activity. Mean ovary weights for various size classes presented in Table 4 indicate that ovary weights were highest from May to July (0.01-0.29 g) and lowest from November to April (0.01-0.08 g). As judged by gonadal weights and egg maturity, spawning apparently occurs from May to August in the North Fork Holston River.

Assuming that all mature eggs produced during the spawning season by a gravid female are laid (Winn 1958), egg numbers for redline darters in this study (23 to 131) are lower than those reported for other darter species (Winn 1958; Bryant 1979; Burr and Page 1979; Lindquist et al. 1981). Winn (1958) reported that in several species females spawn with different males and lay only a few eggs at each spawning. Female redline darters have been observed burying themselves in the gravel several times during spawning, laying several eggs at a time (Stiles 1972). Females may already have laid a portion of their eggs before collection in summer, and egg numbers reported here may not represent total numbers of eggs laid during the spawning season. The unusually low correlation between total length of females and number of eggs tends to support that conclusion.

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